

What is Claimed:

1. A sensor chip comprising:
a fluorescence quenching surface;
5 a first nucleic acid molecule comprising first and second ends with the first end bound to the fluorescence quenching surface, a first region, and a second region complementary to the first region, the first nucleic acid molecule having, under appropriate conditions, either a hairpin conformation with the first and second regions hybridized together or a non-hairpin conformation; and
10 a first fluorophore bound to the second end of the first nucleic acid molecule;
whereby when the first nucleic acid molecule is in the hairpin conformation, the fluorescence quenching surface substantially quenches fluorescent emissions by the first fluorophore, and when the first nucleic acid molecule is in the
15 non-hairpin conformation fluorescent emissions by the fluorophore are substantially free of quenching by the fluorescence quenching surface.
2. The sensor chip according to claim 1, wherein the fluorescence quenching surface is present on a substrate.
- 20 3. The sensor chip according to claim 2 wherein the fluorescence quenching surface is present over substantially the entire substrate.
4. The sensor chip according to claim 2 wherein the fluorescence
25 quenching surface is present in a plurality of discrete locations on the substrate.
5. The sensor chip according to claim 1 wherein the fluorescence quenching surface is formed of a conductive metal or metal alloy.
- 30 6. The sensor chip according to claim 5 wherein the conductive metal or metal alloy is selected from the group of gold, silver, platinum, copper, cobalt, iron, iron-platinum, and aluminum.
7. The sensor chip according to claim 1 wherein the fluorescence
35 quenching surface is formed of a semiconductor material.

8. The sensor chip according to claim 7 wherein the semiconductor material is selected from the group of undoped silicon, p-doped silicon, n-doped silicon, alloys of undoped, p-doped or n-doped silicon, semiconductor materials based on Group III element nitrides, and mixtures thereof.

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9. The sensor chip according to claim 1 wherein the fluorophore is a dye, a protein, or a semiconductor nanocrystal.

10. The sensor chip according to claim 9 wherein the fluorophore is a dye selected from the group of Cy2TM, YO-PROTM-1, YOYOTM-1, Calcein, FITC, FluorXTM, AlexaTM, Rhodamine 110, 5-FAM, Oregon GreenTM 500, Oregon GreenTM 488, RiboGreenTM, Rhodamine GreenTM, Rhodamine 123, Magnesium GreenTM, Calcium GreenTM, TO-PROTM-1, TOTO[®]-1, JOE, BODIPY[®] 530/550, DiI, BODIPY[®] TMR, BODIPY[®] 558/568, BODIPY[®] 564/570, Cy3TM, AlexaTM 546, TRITC, 15 Magnesium OrangeTM, Phycoerythrin R&B, Rhodamine Phalloidin, Calcium OrangeTM, Pyronin Y, Rhodamine B, TAMRA, Rhodamine RedTM, Cy3.5TM, ROX, Calcium CrimsonTM, AlexaTM 594, Texas Red[®], Nile Red, YO-PROTM-3, YOYOTM-3, R-phycocyanin, C-Phycocyanin, TO-PROTM-3, TOTO[®]-3, DiD DiI(5), Cy5TM, Thiadicarbocyanine, and Cy5.5TM.

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11. The sensor chip according to claim 9 wherein the fluorophore is a protein selected from the group of green fluorescent proteins, blue fluorescent proteins, and phycobiliproteins.

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12. The sensor chip according to claim 9 wherein the fluorophore is a semiconductor nanocrystal formed of one or more semiconductor materials.

13. The sensor chip according to claim 12 wherein the semiconductor nanocrystal comprises a core formed of a first semiconductor material and a shell surrounding the core formed of a second semiconductor material. 30

14. The sensor chip according to claim 1 wherein the first nucleic acid molecule is DNA.

15. The sensor chip according to claim 14 wherein the first nucleic acid molecule comprises one or more modified bases.

16. The sensor chip according to claim 1 wherein the first nucleic acid molecule is a peptide nucleic acid.

17. The sensor chip according to claim 1 wherein the first and second regions of the first nucleic acid molecule are at least about four nucleotides in length.

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18. The sensor chip according to claim 1 further comprising:
a second nucleic acid molecule different from the first nucleic acid molecule and comprising first and second ends with the first end bound to the fluorescence quenching surface, a first region, and a second region complementary to the first region, the second nucleic acid molecule having, under appropriate conditions, either a hairpin conformation with the first and second regions hybridized together or a non-hairpin conformation; and

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a second fluorophore bound to the second end of the second nucleic acid molecule;

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whereby when the second nucleic acid molecule is in the hairpin conformation, the fluorescence quenching surface substantially quenches fluorescent emissions by the second fluorophore, and when the second nucleic acid molecule is in the non-hairpin conformation, fluorescent emissions by the second fluorophore are substantially free of quenching by the fluorescence quenching

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surface.

19. The sensor chip according to claim 18 wherein the first and second nucleic acid molecules are bound to the fluorescence quenching surface in discrete first and second locations, respectively.

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20. The sensor chip according to claim 19 wherein the first and second fluorophores are the same or different.

21. The sensor chip according to claim 18 wherein the first and second nucleic acid molecules are both bound to the fluorescence quenching surface in a single location.

5 22. The sensor chip according to claim 21 wherein the first and second fluorophores are different.

23. The sensor chip according to claim 22 wherein the fluorescent emissions of the first and second fluorophores are characterized by emission maxima
10 that are spectrally separated by at least about 1 nm.

24. The sensor chip according to claim 1 further comprising:
one or more additional nucleic acid molecules each comprising first and second ends with the first end bound to the fluorescence quenching surface,
15 a first region, and a second region complementary to the first region, each of the one or more additional nucleic acid molecules having, under appropriate conditions, either a hairpin conformation with the first and second regions hybridized together or non-hairpin conformation; and

one or more additional fluorophores bound, respectively, to
20 second ends of the one or more additional nucleic acid molecules;
whereby when any of the one or more additional nucleic acid molecules is in the hairpin conformation, the fluorescence quenching surface substantially quenches fluorescent emissions by the fluorophore attached to its second end, and when any of the one or more additional nucleic acid molecules is in the non-
25 hairpin conformation, fluorescent emissions by the fluorophore attached to its second end is substantially free of quenching by the fluorescence quenching surface.

25. The sensor chip according to claim 1 wherein the first nucleic acid molecule has the nucleotide sequence of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID
30 NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, or SEQ ID NO: 13.

26. The sensor chip according to claim 1 wherein the sensor chip comprises a plurality of the first nucleic acid molecules, the sensor chip further
35 comprising:

a plurality of spacer molecules bound to the fluorescence quenching surface, thereby inhibiting interaction between adjacent first nucleic acid molecules.

27. The sensor chip according to claim 26 wherein the ratio of
5 spacer molecules to first nucleic acid molecules is between about 2:1 up to about 18:1.

28. The sensor chip according to claim 26 wherein the ratio of
spacer molecules to first nucleic acid molecules is between about 5:1 up to about 15:1.

10 29. A biological sensor device comprising:
a sensor chip according to claim 1;
a light source that illuminates the sensor chip at a wavelength
suitable to induce fluorescent emissions by the first fluorophore; and
a detector positioned to detect fluorescent emissions by the first
15 fluorophore.

30. The biological sensor device according to claim 29 wherein the
light source is a laser or an arc lamp.

20 31. The biological sensor device according to claim 30 wherein the
wavelength is between about 200 nm and 2000 nm.

32. The biological sensor device according to claim 29 wherein the
detector is a charge coupled device, a photomultiplier tube, an avalanche photodiode,
25 or a photodiode.

33. The biological sensor device according to claim 29 further
comprising:
a notch filter positioned between the light source and the sensor
30 chip.

34. The biological sensor device according to claim 29 further
comprising:
a bandpass filter positioned between the sensor chip and the
35 detector.

35. The biological sensor device according to claim 34 wherein the bandpass filter allows passage of light within a range that is not more than about 10 nm greater or less than the wavelength of the maximum emissions of the first fluorophore.

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36. The biological sensor device according to claim 29 further comprising:

an inverted microscope comprising an objective lens and a stage upon which the sensor chip is mounted.

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37. A nucleic acid probe comprising first and second ends, the first end being modified for coupling to a surface and the second end being bound to a fluorophore, the nucleic acid probe further comprising a first region, and a second region complementary to the first region, wherein, under appropriate conditions, the nucleic acid probe has either a hairpin conformation with the first and second regions hybridized together or a non-hairpin conformation, with one or both of the first and second regions being adapted for hybridization to a target nucleic acid molecule.

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38. The nucleic acid probe according to claim 37 wherein the nucleic acid is RNA.

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39. The nucleic acid probe according to claim 37 wherein the nucleic acid is DNA.

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40. The nucleic acid probe according to claim 37 wherein the nucleic acid is PNA.

41. The nucleic acid probe according to claim 37 wherein the first end comprises a C6 thiol-modified base.

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42. The nucleic acid probe according to claim 37 wherein the fluorophore is a dye, a protein, or a semiconductor nanocrystal.

43. The nucleic acid probe according to claim 37 comprising the nucleotide sequence of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, or SEQ ID NO: 13.

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44. A method of detecting the presence of a target nucleic acid molecule in a sample comprising:

exposing the sensor chip according to claim 1 to a sample under conditions effective to allow any target nucleic acid molecule in the sample to
10 hybridize to the first and/or second regions of the first nucleic acid molecule;

illuminating the sensor chip with light sufficient to cause emission of fluorescence by the first fluorophore; and

determining whether or not the sensor chip emits fluorescent emissions of the first fluorophore upon said illuminating, wherein fluorescent
15 emission by the sensor chip indicates that the first nucleic acid molecule is in the non-hairpin conformation and therefore that the target nucleic acid molecule is present in the sample.

45. The method according to claim 44 wherein said illuminating is
20 carried out with a laser.

46. The method according to claim 44 wherein light emitted by the laser is passed through a notch filter prior to reaching the sensor chip.

25 47. The method according to claim 44 wherein said determining comprises collecting fluorescent emission from the sensor chip using a charge coupled device.

30 48. The method according to claim 44 further comprising:
passing fluorescent emissions through a bandpass filter prior to said collecting.

49. A method of genetic screening comprising:
performing the method according to claim 44 with a sensor chip
having a first nucleic acid molecule with the first and/or second region thereof specific
for hybridization with a first genetic marker.

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50. The method according to claim 49 wherein the genetic marker
is associated with a disease state or contains a polymorphism.

51. The method according to claim 49 wherein the sensor chip
10 further comprises:

one or more additional nucleic acid molecules each comprising
first and second ends with the first end bound to the fluorescence quenching surface,
a first region, and a second region complementary to the first region, each of the one
or more additional nucleic acid molecules having, under appropriate conditions, either
15 a hairpin conformation with the first and second regions thereof hybridized together or
a non-hairpin conformation; and

one or more additional fluorophores bound, respectively, to
second ends of the one or more additional nucleic acid molecules, whereby when any
of the one or more additional nucleic acid molecules is in the hairpin conformation,
20 the fluorescence quenching surface substantially quenches fluorescent emissions by
the fluorophore attached to its second end, and when any of the one or more additional
nucleic acid molecules is in the non-hairpin conformation fluorescent emissions by the
fluorophore attached to its second end is substantially free of quenching by the
fluorescence quenching surface.

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52. The method according to claim 51 wherein each of the one or
more additional nucleic acid molecules is associated with a distinct genetic marker.

53. A method of detecting presence of a pathogen in a sample
30 comprising:

performing the method according to claim 44 with a sensor chip
having a first nucleic acid molecule with at least portions of the first and/or second
region thereof specific for hybridization with a target nucleic acid molecule of a
pathogen.

54. The method according to claim 53 wherein the pathogen is a bacteria, a virus, a fungus, or a parasite.

55. The method according to claim 53 wherein the pathogen is a
5 bacteria selected from the group of *Acinetobacter calcoaceticus*, *Actinobacillus*
species, *Aeromonas hydrophila*, *Amycolata autotrophica*, *Arizona hinshawii*, *Bacillus*
anthracis, *Bartonella* species, *Brucella* species, *Bordetella* species, *Borrelia* species,
Campylobacter species, *Chlamydia* species, *Clostridium* species, *Corynebacterium*
species, *Dermatophilus congolensis*, *Edwardsiella tarda*, *Erysipelothrix insidiosa*,
10 *Escherichia coli*, *Francisella tularensis*, *Haemophilus* species, *Klebsiella* species,
Legionella pneumophila, *Leptospira interrogans*, *Listeria* species, *Moraxella* species,
Mycobacteria species, *Mycobacterium avium*, *Mycoplasma* species, *Neisseria* species,
Nocardia species, *Pasteurella* species, *Pseudomonas* species, *Rhodococcus equi*,
Salmonella species, *Shigella* species, *Sphaerophorus necrophorus*, *Staphylococcus*
15 *aureus*, *Streptobacillus moniliformis*, *Streptococcus* species, *Treponema* species,
Vibrio species, and *Yersinia* species.

56. The method according to claim 53 wherein the pathogen is a
virus selected from the group of Adenoviruses, Cache Valley virus, Coronaviruses,
20 Coxsackie A and B viruses, Cytomegaloviruses, Echoviruses, Encephalomyocarditis
virus (EMC), Flanders virus, Hart Park virus, Hepatitis viruses-associated antigen
material, Herpesviruses, Influenza viruses, Langat virus, Lymphogranuloma venereum
agent, Measles virus, Mumps virus, Parainfluenza virus, Polioviruses, Poxviruses,
Rabies virus, Reoviruses, Respiratory syncytial virus, Rhinoviruses, Rubella virus,
25 Simian viruses, Sindbis virus, Tensaw virus, Turlock virus, Vaccinia virus, Varicella
virus, Vesicular stomatitis virus, Vole rickettsia, Yellow fever virus, Avian leukosis
virus, Bovine leukemia virus, Bovine papilloma virus, Chick-embryo-lethal orphan
(CELO) virus or fowl adenovirus 1, Dog sarcoma virus, Guinea pig herpes virus,
Lucke (Frog) virus, Hamster leukemia virus, Marek's disease virus, Mason-Pfizer
30 monkey virus, Mouse mammary tumor virus, Murine leukemia virus, Murine sarcoma
virus, Polyoma virus, Rat leukemia virus, Rous sarcoma virus, Shope fibroma virus,
Shope papilloma virus, Simian virus 40 (SV-40), Epstein-Barr virus (EBV), Feline
leukemia virus (FeLV), Feline sarcoma virus (FeSV), Gibbon leukemia virus (GaLV),
Herpesvirus (HV) ateles, Herpesvirus (HV) saimiri, Simian sarcoma virus (SSV)-1,

Yaba, Monkey pox virus, Arboviruses, Dengue virus, Lymphocytic choriomeningitis virus (LCM), Rickettsia, Yellow fever virus, Ebola fever virus, Hemorrhagic fever agents, Herpesvirus simiae, Lassa virus, Marburg virus, Tick-borne encephalitis virus complex, and Venezuelan equine encephalitis virus.

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57. The method according to claim 53 wherein the pathogen is a fungus selected from the group of *Blastomyces dermatitidis*, *Cryptococcus neoformans*, *Paracoccidioides braziliensis*, *Trypanosoma cruzi*, *Coccidioides immitis*, *Pneumocystis carinii*, and *Histoplasma* species.

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58. The method according to claim 53 wherein the pathogen is a parasite selected from the group of *Endamoeba histolytica*, *Leishmania* species (all), *Naegleria gruberi*, *Schistosoma mansoni*, *Toxocara canis*, *Toxoplasma gondii*, *Trichinella spiralis*, and *Trypanosoma cruzi*.

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59. The method according to claim 53 wherein the sensor chip further comprises:

one or more additional nucleic acid molecules each comprising first and second ends with the first end bound to the fluorescence quenching surface, a first region, and a second region complementary to the first region, each of the one or more additional nucleic acid molecules having, under appropriate conditions, either a hairpin conformation with the first and second regions thereof hybridized together or a non-hairpin conformation; and

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one or more additional fluorophores bound, respectively, to second ends of the one or more additional nucleic acid molecules, whereby when any of the one or more additional nucleic acid molecules is in the hairpin conformation, the fluorescence quenching surface substantially quenches fluorescent emissions by the fluorophore attached to its second end, and when any of the one or more additional nucleic acid molecules is in the non-hairpin conformation fluorescent emissions by the fluorophore attached to its second end is substantially free of quenching by the fluorescence quenching surface.

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60. The method according to claim 59 wherein the first nucleic acid molecule hybridizes to the target nucleic acid molecule from a first pathogen and the one or more additional nucleic acid molecules hybridize to one or more additional target nucleic acid molecules, respectively, also from the first pathogen.

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61. The method according to claim 59 wherein the first nucleic acid molecule hybridizes to the target nucleic acid molecule from a first pathogen and the one or more additional nucleic acid molecules hybridize to one or more additional target nucleic acid molecules, respectively, from one or more pathogens distinct of the first pathogen.

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62. A method of making a sensor chip, the method comprising:
providing a fluorescence quenching surface;
exposing the fluorescence quenching surface to a plurality of
first nucleic acid molecules each comprising first and second ends with the first end
being modified for coupling to the fluorescence quenching surface, a first region, and
a second region complementary to the first region, and each first nucleic acid
molecule having, under appropriate conditions, either a hairpin conformation with the
first and second regions hybridized together or a non-hairpin conformation; and
exposing the fluorescence quenching surface to a plurality of
spacer molecules each including a reactive group capable of coupling to the
fluorescence quenching surface, whereby the plurality of spacer molecules, when
bound to the fluorescence quenching surface, inhibit interaction between adjacent first
nucleic acid molecules bound to the fluorescence quenching surface.

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63. The method according to claim 62 wherein said exposing to the spacer molecules is carried out prior to said exposing to the first nucleic acid molecules.

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64. The method according to claim 62 wherein said exposing to the spacer molecules and said exposing to the first nucleic acid molecules are carried out simultaneously.

65. The method according to claim 62 wherein the ratio of spacer molecules to first nucleic acid molecules is between about 2:1 up to about 18:1.

66. The method according to claim 65 wherein the ratio of spacer
5 molecules to first nucleic acid molecules is between about 5:1 up to about 15:1.